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A reduced size of the ovarian follicle pool is associated with an increased risk of a trisomic pregnancy in IVF-treated women

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BACKGROUND: The increased risk of a trisomic pregnancy with a woman's age arises from an increased rate of meiotic non-disjunction in the oocytes. It has been hypothesized that the increase in meiotic errors is related to the decreasing number of oocytes with age. Our aim was to assess the relation between trisomic pregnancy and three parameters of oocyte quantity.

METHODS: In a Dutch nationwide database on *in vitro* fertilization (IVF) treatment from 1983 to 1995, we identified 28 women with a trisomic pregnancy conceived via or within 1 year from IVF treatment. We selected five age-matched controls with a healthy child for each trisomy case. We performed a case–control study to examine whether trisomy cases more often had a history of ovarian surgery and a lower response to ovarian hyperstimulation than controls. Subsequently, cases and controls were followed to compare the incidence of signs of menopause at the end of the study period as self-reported by questionnaire.

RESULTS: Logistic regression analysis showed an association between trisomic pregnancy and a history of ovarian surgery [odds ratio (OR) 3.3; 95% confidence interval (CI): 1.0–10.5; $P = 0.04$] and between trisomic pregnancy and retrieval of $\leq$4 oocytes during IVF treatment (OR 4.0; 95% CI: 1.4–11.5; $P = 0.01$). The adjusted OR for signs of menopause associated with trisomic pregnancy was 5.7 (95% CI: 1.1–29.9; $P = 0.04$).

CONCLUSIONS: Our results suggest that IVF-treated women with a reduced ovarian follicle pool are at increased risk of a trisomic pregnancy, independent of their age. Our findings support the hypothesis that follicle pool size and not chronological age determines a woman's trisomy risk. Since a questionnaire was used, we cannot fully exclude the possibility of selection bias in this study.

Key words: trisomic pregnancy / trisomy / ovarian reserve / poor response / *in vitro* fertilization

Introduction

The increased risk of a trisomic pregnancy with a woman's age arises from an increased rate of meiotic non-disjunction in the oocytes (Hassold *et al.*, 1996; Eichenlaub-Ritter, 1998). It has been hypothesized that this increase in meiotic errors is related to the decreasing number of oocytes with age (Henderson and Edwards, 1968; Brook *et al.*, 1984; Warburton, 1989, 2005; Zheng and Byers, 1992). Selection of oocytes may become impaired when less oocytes are available (limited pool hypothesis) or physiological

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changes accompanying follicle loss, such as the increase in follicle-stimulating hormone, may affect oocyte integrity (Warburton, 1989, 2005; Dursun *et al*., 2005; McTavish *et al*., 2007).

The quantitative size of the ovarian follicle pool is inversely related to age, but shows substantial variation among peers (Te Velde and Pearson, 2002). This inter-individual variation in follicle pool size is reflected in the wide age range for the onset of menopause, the event that indicates imminent depletion of the follicle pool (Te Velde *et al*., 1998). In addition to ageing, iatrogenic intervention, such as ovarian surgery, may also reduce the follicle pool. The size of the follicle pool during the fertile period can be estimated by so-called ovarian reserve tests, i.e. hormonal or sonographic tests, such as follicle-stimulating hormone (FSH), anti-Müllerian hormone or antral follicle count (Broekmans *et al*., 1998, 2006). The number of follicles that develop in response to ovarian hyperstimulation during *in vitro* fertilization (IVF) treatment can be regarded as a dynamic ovarian reserve test (Broekmans *et al*., 2006).

If indeed the effect of maternal age on trisomy risk is explained by the decrease in follicle number, one would expect to find an association between the above parameters of oocyte quantity and trisomy risk, independent of female age. We addressed this issue in a Dutch nationwide cohort of women undergoing IVF treatment. We hypothesized that the risk of a trisomic pregnancy is elevated in women with a reduced size of the ovarian follicle pool, independent of their age. Therefore, we performed a case–control study to examine whether women with a trisomic pregnancy more often had a history of ovarian surgery and a lower response to ovarian hyperstimulation. Subsequently, we followed these women to assess whether the trisomy cases had more often reached menopausal transition or menopause at the end of the study period than the controls.

Materials and Methods

The present study is a part of the OMEGA project, a large retrospective cohort study in the Netherlands originally designed to assess the effects of ovarian hyperstimulation in IVF treatment on the risk of hormone-related cancers. The study population, study procedures and data collection methods have been described in detail elsewhere (De Boer *et al*., 2003; Klip *et al*., 2003). In brief, all subfertile women starting at least one IVF cycle in the Netherlands between 1 January 1983 (the national start of IVF treatment) and 1 January 1995 were retrospectively included in the cohort ($n = 19\,840$). The institutional review boards from all the participating IVF centres approved the study protocol. From 1997 to 1999, questionnaires on risk factors were sent to all women who could be traced, including detailed questions on reproductive history, lifestyle and health problems in their children (response rate of 71%). In a form attached to the questionnaire, women could give their permission to search their medical files. Trained research assistants retrieved data on medical and reproductive history, subfertility characteristics and course and outcome of treatment from the medical files of the women who had given written permission to do so. Owing to limited project funding data collection could only be completed for 75% of these women. The women with missing medical file data did not represent a subgroup with files or data that were for any reason especially hard to retrieve; data collection was completed per participating IVF clinic before moving on to the next clinic and therefore from some clinics no data were collected at all.

We used a case–control design to assess the association between ovarian surgery and ovarian response to IVF treatment and trisomic pregnancy. Cases were women with a trisomic pregnancy; controls were women with a live birth without a trisomy. Subsequently, we followed these women to examine whether cases with a trisomic pregnancy had an increased risk of natural menopause or menopausal transition at the end of the study period compared with controls. We searched for cases of trisomic pregnancy in the complete OMEGA database. In data from both medical files and questionnaires, we assessed the variables reporting pregnancy outcome, including open fields with comments on pregnancies and health problems in children. We selected all women with comments possibly related to trisomic pregnancy and all those with an induced abortion or stillbirth for which no explanation was recorded. For all selected women who had given their written permission to do so, we searched the original medical records to see if a trisomic pregnancy had been documented; in total 215 medical files were searched. Trisomic pregnancies, confirmed by karyotype, were selected for analysis if the pregnancy resulted from IVF treatment or was naturally conceived within a year before or after IVF treatment. We decided beforehand to include naturally conceived trisomic pregnancies in order to maximize our sample size. We chose an (arbitrary) cut-off of 1 year, assuming that the ovarian response at the nearest IVF treatment would be representative for the ovarian status at the time of conception within 1 year. Sex chromosomal aneuploidies were excluded. In total, we identified 28 cases of trisomic pregnancy fulfilling our criteria. Next to non-disjunction in the oocyte, in a minority of cases (<10%) trisomies may be caused by an unbalanced translocation or may be of paternal origin (Freeman *et al*., 2007). We did not confirm the origin of the trisomic pregnancies in this study and no information on the karyotype of the couples was available. None of the women contributed more than one case of trisomic pregnancy.

For each trisomy case, we selected five individually matched controls who did not have a trisomic pregnancy from the same database. The controls were women who conceived via IVF treatment or within a year before or after IVF treatment and had a live birth. They were matched for age at the time of IVF treatment, mode of conception, period of IVF treatment and fertility centre. The IVF cycle resulting in the trisomic or control pregnancy or, in the case of natural conception, the IVF cycle nearest to the conception of the trisomic or control pregnancy is hereafter referred to as the 'index IVF cycle'.

For cases and controls, we retrieved subfertility and lifestyle characteristics from questionnaire and medical file data. Data on ovarian surgery were compared with the data from the Dutch nationwide network and registry of histo- and cytopathology (PALGA) for confirmation, where possible. Ovarian surgery was recorded only if performed before the start of the index IVF cycle. Ovarian response during the index IVF cycle was defined as the number of oocytes retrieved at follicle aspiration. A poor response was defined as an oocyte yield of 0–3 oocytes. In previous studies employing this frequently used cut-off value, it was shown that women with a poor response have an increased risk of early menopause (De Boer *et al*., 2002, 2003; Lawson *et al*., 2003). Age was defined as the woman's age at the start of the index IVF cycle. For body mass index and smoking habits, we used the self-reported values in the questionnaire. Total medication dose was defined as the total number of ampoules of recombinant FSH or human menopausal gonadotrophin used in the index IVF cycle. We had to replace 12 of the original controls, since 11 controls had an unknown response to the index IVF cycle and 1 control reported a post-partum death without explanation and had not given permission to search the medical file.

For the second analysis, we studied data from the questionnaire on the length and regularity of the menstrual cycle at the first visit to the fertility clinic and at the moment of filling in the questionnaire. The median time

between the index IVF cycle and filling in the questionnaire was 4.1 years (range 0.6–11.9 years). We selected the women who reported a regular cycle (i.e. next cycle predictable within 4 days) with a cycle length 21–35 days at their first visit to the fertility clinic. Next, we determined their menopausal status at the time of the questionnaire. Natural menopause was defined as the spontaneous absence of vaginal bleeding for at least 1 year, except if due to pregnancy or breastfeeding. Menopausal transition was defined as a change in cycle pattern to an irregular cycle (i.e. next cycle not predictable within 4 days) and/or a change in cycle length to <21 or >35 days. Those whose last menstruation dated 3–11 months before completion of the questionnaire were also regarded as being in menopausal transition (if not pregnant or breastfeeding). The use of hormonal replacement therapy (HRT) was considered as a sign of menopause, as its use in the Netherlands is generally restricted to women after menopause (De Jong-van den Berg *et al*., 2006). Women with natural menopause, menopausal transition and use of HRT were combined into one category having 'signs of menopause', whereas those reporting a regular cycle between 21 and 35 days at the time of the questionnaire were considered premenopausal. Women using oral contraceptives at the time of the questionnaire were excluded from analysis, as were women with a history of hysterectomy or chemo- or radiotherapy.

The original matching between cases and controls was not taken into account for the analysis on menopausal status. Cases and controls were independently selected for this analysis if their data were complete and they met our inclusion criteria.

We used multivariate conditional logistic regression analysis to calculate the odds ratios (ORs) for trisomic pregnancy associated with a history of ovarian surgery and response to IVF treatment. We analysed the variable ovarian response in three different ways, i.e. linear, ordinal and dichotomous, in order to be able to identify both a possible linear or non-linear relation. Response to IVF treatment was divided into three categories (tertiles); smaller categories could result in inaccurate estimates due to limited sample size. Ovarian response was dichotomized into poor response yes or no. Values for smoking habits, body mass index and total medication dose were not known for all cases and controls. If a value for a case was missing, the case and its matched controls were removed from the analysis. If a value for a control was missing, it was assigned the value of the respective case; thus the control concerned did not contribute to the risk estimate (Rookus and Van Leeuwen, 1994). Each potential confounder was added separately to the logistic model. If this resulted in a change in OR of 10% or more, the confounder was included in the model (Rothman and Greenland, 1998). This process was repeated until no other confounders were identified.

Second, we used unconditional logistic regression analysis to examine whether women with a trisomic pregnancy more often showed signs of menopause at the time of filling in the questionnaire at the end of the study period than controls. This analysis may be regarded as a follow-up study of the case–control cohort, defined by clear eligibility criteria. Matching is not relevant in this kind of analysis and was not taken into account. We calculated the OR for signs of menopause associated with trisomic pregnancy and evaluated potential confounders as described above. A history of ovarian surgery and response to IVF treatment were not regarded as potential confounders because both parameters are related to age at menopause (Melica *et al*., 1995; De Boer *et al*., 2003; Lawson *et al*., 2003). Since we hypothesized that women with a history of ovarian surgery or a low response to IVF treatment have an increased risk of trisomic pregnancy, correction for these variables would lead to underestimating the true relation between trisomic pregnancy and signs of menopause. A *P*-value of <0.05 was considered statistically significant. Data were analysed with SPSS 14.0 (SPSS Inc., Chicago, IL, USA) and EGRET 2.0.31 (Cytel Software, Cambridge, MA, USA).

Results

In total, we identified 28 cases of trisomic pregnancy conceived via or within 1 year before or after IVF treatment. For each case, five controls were selected ($n = 140$). Median age of the cases at the time of the index IVF cycle was 37.9 years. Among the trisomic pregnancies were 24 cases of trisomy 21, 3 cases of trisomy 18 and 1 case of trisomy 13. In 8 cases the pregnancy resulted in a live birth (all trisomy 21), in 2 cases a stillbirth was reported and 18 trisomic pregnancies were terminated after prenatal diagnosis. In four cases, the fetus with trisomy was one of dizygotic twins. In 26 cases, the trisomic pregnancy followed IVF treatment, whereas two trisomic pregnancies resulted from spontaneous conception.

Parameters of oocyte quantity, subfertility and lifestyle characteristics of cases and controls and the ORs for trisomic pregnancy are shown in Table I. Details on ovarian surgery are given in Table II. The OR for trisomic pregnancy associated with a history of ovarian surgery was 3.3 [95% confidence interval (CI): 1.0–10.5; $P = 0.04$]. Adding the variables body mass index, smoking habits, total medication dose and multiple pregnancy to the regression model did not materially affect the OR.

The OR for trisomic pregnancy associated with ≤4 retrieved oocytes was 4.0 (95% CI: 1.4–11.5; $P = 0.01$; reference category ≥5 oocytes). The OR for trisomic pregnancy associated with poor response (≤3 retrieved oocytes) was 2.7 (95% CI: 0.7–10.7; $P = 0.15$). Adding the potential confounders did not substantially change the ORs for trisomic pregnancy associated with oocyte number.

Figure 1 shows the flowchart of cases and controls suitable for analysis of menopausal status. Of the original 168 women, 72 met our criteria. The proportion of cases with complete data on menopausal status (12 of 28; 43%) was the same as the proportion of controls available for this analysis (60 of 140; 43%). Median age at the time of filling in the questionnaire was 42.4 years (range 33.3–51.4 years). Of the nine women with signs of menopause, four were cases (33%) and five controls (8.3%). The unadjusted OR for signs of menopause associated with trisomic pregnancy was 5.5 (95% CI: 1.2–24.9; $P = 0.03$). Age and smoking habits at the time of completing the questionnaire were identified as confounders. The adjusted OR for signs of menopause associated with trisomic pregnancy was 5.7 (95% CI: 1.1–29.9; $P = 0.04$).

All analyses were repeated using the 12 original controls that had been replaced and showed comparable results. Similarly, the exclusion of the two naturally conceived cases and their controls did not materially affect the odds ratios; if excluded from the analysis, the OR for trisomic pregnancy associated with ovarian surgery was 2.6 (95% CI: 0.8–9.0; $P = 0.13$), the OR for trisomic pregnancy associated with ≤4 retrieved oocytes was 4.9 (95% CI: 1.6–15.2; $P < 0.01$) and the adjusted OR for signs of menopause associated with trisomic pregnancy was 8.6 (95% CI: 1.4–52.4, $P = 0.02$).

Discussion

The results of our study point to a relation between trisomic pregnancy and reduced follicle pool size, independent of a woman's age. Trisomy cases more often had a history of ovarian surgery and low ovarian response to IVF treatment and more often showed signs of menopause at follow-up than controls.

Table I ORs of trisomic pregnancy associated with parameters of oocyte quantity, subfertility and lifestyle characteristics

	n	Cases[a] [*n* = 28; median (10th–90th percentile) or no. (%)]	Controls[a] [*n* = 140; median (10th–90th percentile) or no. (%)]	OR for trisomic pregnancy (95% CI)[b]	*P*-value
Parameters of oocyte quantity					
History of ovarian surgery before IVF cycle	168				
Yes		5 (17.9)	7 (5.7)	3.3 (1.0–10.5)	0.04
No		23 (82.1)	133 (94.3)	1.0 (reference)	—
Total number of oocytes retrieved in IVF cycle	168	6.5 (2–19)	8 (4–18)	1.0 (0.9–1.0)	0.32
Number of retrieved oocytes in categories	168				
1–4		9 (32.2)	17 (12.1)	3.7 (1.2–11.7)	0.03
5–8		8 (28.6)	57 (40.7)	0.9 (0.3–2.3)	0.76
≥9		11 (39.3)	66 (47.1)	1.0 (reference)	
Poor response in IVF cycle	168				
Yes (≤3 oocytes)		4 (14.3)	9 (6.4)	2.7 (0.7–10.7)	0.15
No (≥4 oocytes)		24 (85.7)	131 (93.6)	1.0 (reference)	-
Subfertility and lifestyle characteristics					
Smoking at time of IVF treatment	136				
Yes		10 (40.0)	32 (28.8)	1.5 (0.5–3.9)[c]	0.45
No		15 (60.0)	79 (71.2)	1.0 (reference)	
Body mass index	139				
≤25 kg/m^2		19 (76.0)	81 (71.1)	1.0 (reference)	
>25 kg/m^2		6 (24.0)	33 (28.9)	0.6 (0.2–1.6)[c]	0.31
Multiple pregnancy	168				
Yes		4 (14.3)	22 (15.7)	0.9 (0.3–2.8)	0.85
No		24 (85.7)	118 (84.3)	1.0 (reference)	
Total no. of ampoules HMG or rFSH[d]	153	27 (17.5–40.5)	24 (15–40)	1.0 (0.9–1.1)[c]	0.77

[a]Cases and controls were matched for age at the time of IVF treatment, mode of conception, period of IVF treatment and fertility centre.
[b]The ORs shown are crude ORs. No confounders of the associations between trisomic pregnancy and the various parameters of oocyte quantity were identified: adding the variables body mass index, smoking habits, total medication dose and multiple pregnancy to the regression model did not materially affect the ORs.
[c]For these analyses, values of 3 (smoking and body mass index) or 4 cases (medication dose) were unknown; these cases and their matched controls were excluded. Values were unknown for 18 (smoking), 19 (body mass index) and 7 (medication dose) of the remaining controls and substituted by the values of the cases they were matched to.
[d]HMG, human menopausal gonadotrophin; rFSH, recombinant follicle-stimulating hormone.

Table II Characteristics of ovarian surgery

	Type of ovarian surgery	Reason for ovarian surgery	Age at surgery (in years)
Trisomy cases (*n* = 5)	Unilateral adnexal extirpation	Functional cyst	30.0
	Unilateral adnexal extirpation	Unknown	23.1
	Unilateral ovariectomy	Benign cystic teratoma	18.4
	Cystectomy	Endometrioma	34.6
	Cystectomy	Unknown	25.5
Controls (*n* = 8)	Unilateral adnexal extirpation	Tubal-ovarian abscess	29.1
	Unilateral adnexal extirpation	Adhesions	36.2
	Unilateral ovariectomy	Adnexal torsion	33.8
	Cystectomy	Functional cyst	28.9
	Cystectomy	Functional cyst	36.3
	Cystectomy	Functional cyst	32.0
	Cystectomy	Corpus luteum cyst	34.1
	Wedge resection	Polycystic ovarian syndrome	21.9

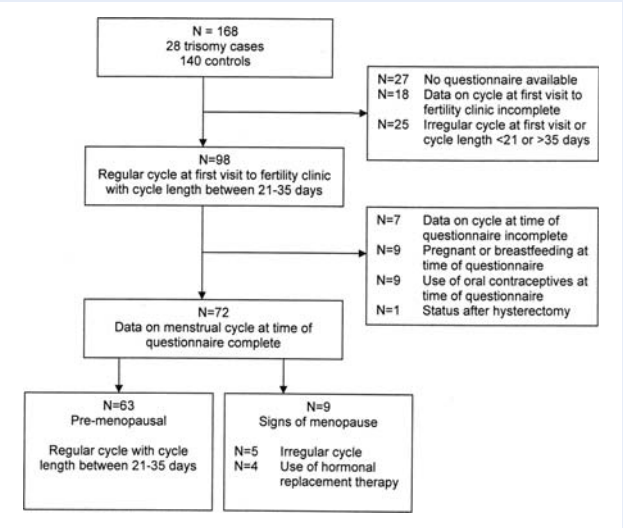

Figure 1 Flow chart of patients eligible for analysis of menopausal status.

The phenomenon of female reproductive ageing is attributed to a decline in both the quantity and the quality of the remaining oocytes (Te Velde and Pearson, 2002; Baird *et al*., 2005). The decrease in oocyte quantity eventually leads to menopause; the decrease in oocyte quality is reflected in the age-related increase in meiotic errors. Loss of oocyte quantity and quality could progress independently of each other. The number of follicles present in the ovaries at any time is most likely determined by the original size of the fetal follicle pool and the rate of atresia of this pool. Oocyte quality may be determined by biological damage accumulating over time. However, some theories do suggest a relation between oocyte quantity and quality. The 'limited pool hypothesis' states that the process of oocyte selection might become impaired if the number of oocytes to select from is decreased (Warburton, 2005). Furthermore, as the number of follicles declines, the endocrine and paracrine environment of the remaining oocytes may hypothetically harm their quality, for example, by increased levels of FSH (Dursun *et al*., 2005; Warburton, 2005; McTavish *et al*., 2007). Our findings suggest a relation between follicle pool size and trisomy risk, independent of a woman's age, and thus support a relation between oocyte quantity and quality. The fact that all three parameters, i.e. ovarian surgery, oocyte number retrieved in IVF treatment and signs of menopause, point towards an increased trisomy risk with reduced follicle pool size is remarkable, as is the fact that, despite the small numbers involved, several associations reached statistical significance.

In this study, a low oocyte yield of $\leq$4 oocytes was statistically significantly associated with an increased risk of a trisomic pregnancy. Interestingly, we did not find a linear association between trisomic pregnancy and number of retrieved oocytes when this was analysed as a continuous variable, nor when it was categorized in tertiles ($\leq$4, 5–8 and $\geq$9 oocytes, respectively). This non-linear relation is not unexpected. For instance, the relation between female age and aneuploidy rate is not linear either, nor is the relation between age and follicle pool size (Hassold and Chiu, 1985; Faddy *et al*., 1992; Hassold and Hunt, 2001; Hansen *et al*., 2008).

Interpretation of the results could be challenged by the limitations of the parameters of oocyte quantity used. We identified a history of ovarian surgery, an iatrogenic depletion of the follicle pool, as a risk factor for trisomic pregnancy. We cannot rule out the possibility that the ovarian pathology leading to the surgery accounted for the decrease in oocyte quality, though the indications for ovarian surgery show a wide variation.

Second, an oocyte yield of $\leq$4 oocytes and an oocyte yield of $\leq$3 oocytes at IVF treatment were both associated with an increased risk of a trisomic pregnancy, but for $\leq$3 oocytes this association was not statistically significant, probably due to the small numbers involved. We considered the OMEGA database to be suitable for this research question despite the fact that medication protocols and laboratory procedures have developed considerably since its inception. No actual improvement of the outcome of IVF treatment in low responders has been shown as a result of these changes (Loutradis *et al*., 2003; Ubaldi *et al*., 2005; Shanbhag *et al*., 2007), and poor response remains one of the chief challenges in today's reproductive medicine.

For our third parameter of oocyte quantity, signs of menopause, we included women with a change in cycle pattern suggesting menopausal transition and women using HRT. We decided beforehand not only to include women that actually reached menopause, since the median time between filling in the questionnaire (from which the data on menopausal status were obtained) and the IVF cycle studied was only 4.1 years. We did not expect a sufficient number of women to be post-menopausal in this short period of time to allow any meaningful analysis: several years before menopause occurs, fertility is already severely impaired, whereas these women actually conceived after the index IVF cycle. However, it turned out that none of the eligible cases and controls had in fact reached natural menopause, which may be regarded as the ultimate parameter for oocyte quantity. The proportion of subjects in our analysis of menopausal status was only 43% of the total study population. In this analysis, the original matching of cases and controls was not taken into account.

We cannot exclude the possibility that the 71% response rate to the questionnaire may have led to selection bias. Trisomic and control pregnancies that were not reported in the questionnaire and not mentioned in the medical file data (e.g. from the non-represented clinics) were missed in the present study. The question is whether women with a trisomic pregnancy and reduced follicle pool size were less or more likely to respond than women with a control pregnancy and reduced follicle pool size. We assume this is not the case since we cannot think of a plausible reason why, but cannot rule out the possibility of selection bias.

Finally, with 28 cases the number of subjects in our matched case–control study was limited, despite the nationwide database of IVF patients available. Fertility is already impaired years before menopause is reached and poor responders in IVF treatment are known to have low pregnancy chances (Saldeen *et al*., 2007). If a low number of remaining oocytes is indeed related to an increased trisomy risk, larger samples will be hard to obtain.

The relation between trisomic pregnancy and parameters of oocyte quantity has been studied previously. Freeman *et al*. (2000) performed a case–control study and found, in line with our results, that women with a child with trisomy 21 significantly more often had a history of ovarian surgery or congenital absence of an ovary compared with controls (7 of 189 cases versus 1 of 329 controls). The findings also

correspond to the mouse studies of Brook *et al*. (1984), which showed an increased incidence of aneuploid embryos in ageing mice after unilateral ovariectomy. Kline *et al*. compared 111 women with a spontaneous miscarriage with a trisomic karyotype with 226 women with a healthy live birth and 157 women with a chromosomally normal pregnancy loss. Compared with the other two groups, the women with a history of trisomic pregnancy entered menopause approximately 1 year earlier (0.96 years, 95% CI: −0.18 to 2.10) (Kline *et al*., 2000). Bartmann *et al*. (2005) found that 104 mothers of a child with Down syndrome entered menopause 0.7 years earlier than 121 control mothers with a healthy child. In both studies, the difference in menopausal age between cases and controls was not statistically significant. To our knowledge, the relation between ovarian response to IVF treatment and trisomy risk has not been studied before.

Well-known parameters for oocyte quantity that were not available in the present study are endocrine and sonographic ovarian reserve tests. Van Montfrans *et al*. (1999, 2002) found that mothers of a child with trisomy 21 ($n = 118$) had significantly higher levels of FSH and lower levels of inhibin B than age-matched controls ($n = 102$). Kline *et al*. did not find statistical differences in antral follicle count, FSH or inhibin B levels between women with a spontaneous miscarriage of a trisomic pregnancy ($n = 54$) compared with women with a spontaneous miscarriage due to other causes ($n = 45$) or with a live birth ($n = 65$) (Kline *et al*., 2004). Nasseri *et al*. karyotyped miscarriage tissue and found higher FSH and/or estradiol levels in women with an aneuploid spontaneous miscarriage ($n = 44$) than in women with a euploid spontaneous miscarriage ($n = 34$) (Nasseri *et al*., 1999). Anti-Müllerian hormone (AMH) is now considered the most promising marker of follicle pool size (Van Rooij *et al*., 2005; Visser *et al*., 2006), but one study comparing AMH levels in samples from a prenatal screening program found no statistically significant difference between 25 women with a Down syndrome pregnancy compared with 125 matched controls (Seifer *et al*., 2007).

In conclusion, despite the absence of general consensus, our results point to a relation between trisomic pregnancy and a reduced size of the ovarian follicle pool, independent of a woman's age. Our findings support a relation between oocyte quantity and quality and indicate that the size of the follicle pool and not chronological age may determine a woman's trisomy risk. Since we used three different parameters of oocyte quantity that all support this relation, this is unlikely to be an accidental finding. However, since a questionnaire was used, we cannot fully exclude the possibility of selection bias. Moreover, results from studies performed in an IVF population cannot simply be extrapolated into the general population. The risk of a trisomic pregnancy for IVF patients may not be comparable to that in the general population, for instance, the effect of embryo selection on trisomic risk is unclear. Our results need confirmation in another IVF cohort, before the clinical use of parameters of oocyte quantity for the assessment of trisomy risk can be considered in IVF patients.

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Appendix

The OMEGA project group includes: R. Schats (Vrije Universiteit Medical Center, Amsterdam), N.S. Macklon (University Medical Center, Utrecht), J.S.E. Laven (Erasmus Medical Center, Rotterdam), C.A.M. Jansen (Diaconessenhuis Voorburg), F.M. Helmerhorst and N. Naaktgeboren (Leiden University Medical Center), B.J. Cohlen (Isala Clinics Zwolle), D.D.M. Braat, J.A.M. Kremer and W.N.P. Willemsen (Radboud University Nijmegen Medical Centre), R.S.G.M. Bots (St Elisabeth Hospital, Tilburg), A.H.M. Simons (University Medical Center, Groningen), F. van der Veen (Academic Medical Center, Amsterdam), J.L.H. Evers (Academic Hospital, Maastricht) and P.A. van Dop (Catharina Hospital, Eindhoven).

Authors' roles

M.L.H.: design of the study, acquisition and analysis of data, main author.

T.M.M.: design of the study, acquisition and analysis of data, critical review.

H.G.: analysis and interpretation of data, critical review.

C.W.B.: interpretation of data, critical review.

C.B.L.: interpretation of data, critical review.

F.J.M.B.: interpretation of data, critical review.

F.E.L.: design of the study, analysis and interpretation of data, critical review.

K.B.: interpretation of data, critical review.

A.H.: design of the study, interpretation of data, critical review.

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